Supporting Information

Biochar impacts soil bacterial community composition and nitrogen cycling in an acidic soil planted with rape

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Main content

Supporting Methods:

Preparation of rape seeding. Picked full seeds were put in 70% alcohol for 30 s and sterilized in 3% (v/v) H_2O_2 for 15 min followed by thorough washing with deionized water, before being germinated in a 37^{o}C thermostat incubator. After 24 hours, uniform seeding were selected and transplanted into pots.

The closed chamber method to measure Nitrous oxide (N_2O) fluxes. The chamber was of the base-lid type, made of polycarbonate engineering plastic. The base was a cylindrical flux chamber of 30 cm in diameter and 90 cm in height. The chamber lid was also made of polycarbonate engineering plastic with the same diameter as the base. When the lid was closed, it compressed a rubber gasket cemented to its underside against a horizontal flange at the top of the base walls, thus providing a gas seal.

Potential nitrification rate and denitrification enzyme activity. Potential nitrification rate (PNR) was measured using a chlorate inhibition method [1]. Briefly, 5.0 g of fresh soil was added to 50-ml centrifuge tubes containing 20 ml phosphate buffer solution (PBS) (g L^{-1}: NaCl, 8.0; KCl, 0.2; Na_2HPO_4, 0.2; NaH_2PO_4, 0.2; pH 7.4) with 1 mM $(NH_4)_2SO_4$. Potassium chlorate with a final concentration of 10 mM was added to the tubes to inhibit nitrite oxidation. The suspension was incubated in a dark incubator at 25°C for 24 h, and nitrite was extracted with 5 ml of 2 M KCl and determined by a spectrophotometer at wavelength of 540 nm with N-(1-naphthyl) ethylenediamine dihydrochloride.

Denitrification enzyme activity was determined using the acetylene (C_2H_2) inhibition technique [2]. Briefly, 10.0 g of fresh soil was added to 120-ml serum bottles containing 10 ml buffer solution (1 mM glucose and 1 mM KNO_3). Flasks were then sealed and made anoxic by repeated evacuation and filling with He for three times. Then C_2H_2 was injected to the headspace and over-pressure was released with a syringe (without piston) to make a final concentration of 10% v/v to inhibit N_2O reduction. All bottles were transferred to the robotized incubation system[3] to monitor N_2O for 5 h. The amount of N_2O in the headspace and DEA were calculated as indicated by Guo *et al*. [2]

Determination of nitrate and ammonium in rape. Rape nitrate and ammonium contents were determined by grinding extraction method: weighted 5 g fresh rape into a mortar, added 1.0 ml 30% trichloroacetic acid and 0.5 g Quartz sand, grinded until the sample was homogenized, transferred the homogenate with 29 ml deionized water to a 50 ml centrifugal tube, and centrifugated for 10 min at a speed of 3000 ×g. Then 2 ml supernatant was diluted to 100 ml and determined by a Continuous Flow Analyser (SAN++, Skalar, Breda, Holland).

Rape nitrite determination. The extraction procedure was described as follows: fresh rape was washed with deionized water, air dried, cut into very small pieces, homogenized with some water (recorded the volume used); 5.00 g homogenate was weighted, washed into a 100 ml flask with 80 ml deionized water, ultrasonic extracted for 30 min (gently shaking the flasks every 5 min to disperse the solid phase), put into 75^{o}C water bath for 5 min; the extraction were took out of the water

bath, cooled to room temperature, diluted to 100 ml, filtered, and centrifugated for 15 min at 8000 ×g. The supernatant was determined by spectrophotometry.

RNA extraction procedure. Briefly, 0.5 g of fresh soil were extracted with 0.5 ml of K_3PO_4 extraction buffer (240 mM, pH 8.0) and 0.5 ml of phenol-chloroform-isoamylalcohol (25:24:1) (pH > 7.8). Samples were lysed for 45 s at the FastPrep-24 speed setting of 5.5 m/s, and the aqueous phase containing nucleic acids was separated by centrifugation at 16,000 × *g* for 5 min at 4°C. The aqueous layer was removed and mixed with an equal volume of chloroform-isoamyl alcohol (24:1). After centrifugation, 2 volumes of 30% (w/v) polyethylene glycol (PEG) 6000 precipitate solution was added to the aqueous phase, incubated at room temperature for 2 h, and then centrifuged for 10 min at 4°C. The nucleic acids are pelleted by centrifugation at 18,000 × *g* at 4°C for 10 min and then washed with ice cold 70% ethanol, air-dried, and resuspended in 50 μl of TE (pH 7.4).

The establishment of standard curves for quantitative PCR. The standard curves for quantitative PCR were established using archaeal and bacterial 16S rRNA, archaeal and bacterial *amoA*, *nirK*, *nirS* and *nosZ* gene fragments cloned into plasmid pGEM-T Easy Vector (3015 bp, Promega, Madison, USA). These gene fragments were amplified with the primers in Table S3, respectively. The amplicons were gel-purified using the Gel Clean-up System (Promega, Madison, USA) and cloned using the pGEM-T Easy cloning kit according to the manufacturer's instructions. Plasmids were transformed into *Escherichia coli* JM109 competent cells. Plasmid DNA was extracted using a Qiagen Plasmid Mini Kit (Qiagen Nordic)

and plasmid concentration was determined using a NanoDrop ND-1000 spectrophotometer. Plasmid DNA was diluted in ten-fold series to generate standard curves.

Cammands used in High-throughput sequencing data preprocessing.

1) pick_otus_through_otu_table.py -i /home/qiime/Desktop/Data/biochar.fna -o /home/ qiime/Desktop/Data/ucr/ -p /home/qiime/Desktop/Data/ucrC_params.txt
2) parallel_identify_chimeric_seqs.py -i /home/qiime/Desktop/Data/ucr/ pynast_aligned_seqs/biochar_rep_set_aligned.fasta -a /home/qiime/qiime_software/ core_set_aligned.fasta.imputed -o /home/qiime/Desktop/Data/ ucr/chimeric_seqs.txt
3) filter_fasta.py -f ucr/pynast_aligned_seqs/biochar_rep_set_aligned.fasta -o ucr/pynast_aligned_seqs/non_chimeric_seqs_rep_set_aligned.fasta -s /home/qiime/Desktop/Data/ ucr/chimeric_seqs.txt -n
4) make_otu_table.py -i ucr/uclust_ref_picked_otus/biochar_otus.txt -o ucr/non_chimeric_otu_table.biom -t ucr/rdp_assigned_taxonomy/ biochar_rep_set_tax_assignments.txt -e /home/qiime/Desktop/Data /ucr/ chimeric_seqs.txt -e ucr/pynast_aligned_seqs/biochar_rep_set_failures.fasta
5) filter_otus_from_otu_table.py -i /home/qiime/Desktop/Data /ucr/non_chimeric_otu_table.biom -o /home/qiime/Desktop/Data /ucr/non_chimeric_otu_table_no_singletons.biom -n 2
6) convert_biom.py -i /home/qiime/Desktop/Data/ ucr/non_chimeric_otu_table_no_singletons.biom -o /home/qiime/Desktop/Data/

ucr/non_chimeric_otu_table_no_singletons.txt -b --header_key taxonomy

7) filter_alignment.py -i ucr/pynast_aligned_seqs /non_chimeric_seqs_rep_set_aligned.fasta -m /home/qiime/Desktop/Data/lanemask_in_1s_and_0s -o ucr/pynast_aligned_seqs

8) make_phylogeny.py -i ucr/pynast_aligned_seqs/non_chimeric_seqs_rep_set_aligned_pfiltered.fasta -o ucr/non_chimeric_rep_set.tre

9) make_otu_heatmap_html.py -i ucr/non_chimeric_otu_table_no_singletons.biom -o ucr/OTU_Heatmap/

10) make_otu_network.py -m /home/qiime/Desktop/Data/ map_biochar.txt -i ucr/non_chimeric_otu_table_no_singletons.biom -o ucr/OTU_Network

11) summarize_taxa_through_plots.py -i ucr/non_chimeric_otu_table_no_singletons.biom -o taxa_summary -m map_biochar.txt

12) per_library_stats.py -i ucr/non_chimeric_otu_table_no_singletons.biom

13) echo "alpha_diversity:metrics shannon,PD_whole_tree,chao1,observed_species,simpson_reciprocal,simpson,simpson_e" > alpha_params.txt

14) alpha_rarefaction.py -i ucr/non_chimeric_otu_table_no_singletons.biom -m map_biochar.txt -o arare -p alpha_params.txt -t ucr/non_chimeric_rep_set.tre -a

15) alpha_rarefaction.py -i ucr/non_chimeric_otu_table_no_singletons.biom -m map_biochar.txt -o arare_19772-p alpha_params.txt -t

ucr/non_chimeric_rep_set.tre -e 19772 -a

16) echo "beta_diversity:metrics " > beta_params.txt

17) beta_diversity_through_plots.py -i ucr/non_chimeric_otu_table_no_singletons.biom -m map_biochar.txt -o bdiv_even_19772/ -t ucr/non_chimeric_rep_set.tre -e 19772-a

Supporting Sequencing Data:

After demultiplexing and quality filtering, 723 854 high-quality sequences were obtained in total, with an average number of sequences per sample of 45 241±2 704 (standard error), ranging from 30 677 to 67 588 sequences in each sample. These sequences were clustered into 40 120 OTUs. Of these OTUs, 59.6% were singletons, accounting for 3.3% of total sequences. High percentages of singletons of classified OTUs are common in metagenomic studies using Illumina sequencing technique.[4, 5] Since the percentage of singleton sequences of the total sequences is low, the sequencing result was reliable. After removing chimeras and singletons, 15 908 OTUs were reserved, with an average of 4685 OTUs per sample. The sequences of chimeras in each sample except sample BP1 varied from 4 to 321. After removing chimeras and singletons, at least 19 772 sequences were obtained for each sample.

Table S1 Basic properties of the soil and biochar used in biochar amendment pot trial study in greenhouse

	pH	EC	Total N ($g\ kg^{-1}$)	Total C ($g\ kg^{-1}$)	C/N	Available P ($mg\ kg^{-1}$)	Available K ($mg\ kg^{-1}$)	Soil texture (%)			Ash content (%)	BET (N_2)[b] surface area (m^2/g)
								Sand	Silt	Clay		
soil	4.48	1.38	2.4	15.4	6.5	118.7	322.1	10.28	78.73	10.99	-	-
Biochar[a]	10.30	5.46	17.8	486	27.3	-	-	-	-	-	29.30	11.97

Note: [a] Rice straw biochar. [b] Measured by an ASAP 2020 surface area and porosity analyzer (Micromeritics, USA).

Table S2 Elemental composition[a] of the soil and biochar used in biochar amendment pot trial study in greenhouse

Element (%)	Si	K	Cl	Ca	Mg	P	Fe	S	Mn	Na	Al	Zn	Rb	Ba	Ti	Cr	Sr
Soil	13.62	10.00	2.60	1.74	0.97	0.93	0.48	0.43	0.34	0.32	0.23	0.067	0.030	0.024	0.023	0.015	0.008
Biochar[b]	29.39	2.13	0.019	0.83	1.11	0.26	4.36	0.098	0.059	0.59	8.27	0.012	0.012	0.059	0.533	0.010	0.012

Note: [a] Determined by X-ray fluorescence spectroscopy with a Panalytical spectrometer (PANalytical B.V., ALMELO, the Neterlands).

[b] Rice straw biochar

Table S3 Primers and PCR conditions used for the real-time PCR in biochar amendment pot trial study

Target group	Primer	Sequence（5'→3'）[b]	Thermal profile for real-time PCR	Reference
Archaeal 16S *rRNA*	A364aF	CGGGGYGCASCAGGCGCGAA	2 min at 94°C , followed by 40 cycles of 20 s at 94°C , 30 s at 63°C , and 32 s at 72°C .	3
	Ar958R	YCCGGCGTTGAVTCCAATT		7
bacterial 16S *rRNA*	1369F	CGGTGAATACGTTCYCGG	2 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C.	8
	1492R	GGWTACCTTGTTACGACTT		
	Probe TM1389F	CTTGTACACACCGCCCGTC		
AOA[a]	Arch-*amoA*F	STAATGGTCTGGCTTAGACG	2 min at 95°C , followed by 40 cycles of 30 s at 95°C , 30 s at 58°C , and 45 s at 72°C .	9
	Arch-*amoA*R	GCGGCCATCCATCTGTATGT		
AOB[a]	*amoA*-1F	GGGGTTTCTACTGGTGGT	2 min at 95°C, followed by 40 cycles of 30 s at 95°C , 30 s at 60°C, and 32 s at 72°C.	10
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
nirK	*nirK*-1F	GGMATGGTKCCSTGGCA	5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 40 s at 58 °C, and 40 s at 72°C.	11
	nirK-5R	GCCTCGATCAGRTTRTGGTT		
nirS	cd3aF	GTSAACGTSAAGGARACSGG	2 min at 95°C, followed by 40 cycles of 5 s at 95°C , 40 s at 62°C, and 35 s at 72°C.	12
	R3cd	GASTTCGGRTGSGTCTTGA		
nosZ	*nosZ*-F	CGYTGTTCMTCGACAGCCAG	1 min at 95°C, followed by 40 cycles of 15 s at 95°C , 15 s at 62°C, and 31 s at 72°C.	13
	nosZ-R	CGSACCTTSTTGCCSTYGCG		

Note: a. AOA-ammonia-oxidizing archaea; AOB-ammonia-oxidizing bacteria. b. Y = C or T; M = A or C; W = A or T; R = A or G; K = G or T; S = C or G.

Table S4 Comparison of α-diversity indices in four treatments with or without biochar amendment

Indices / Treatments	chao1	observed species	PD_whole tree	Shannon	Simpson
C	6337±359b	2659±178c	130.2±5.3c	8.37±0.21c	0.9862±0.0042b
CP	6971±554ab	2947±208bc	144.9±7.4b	8.64±0.16b	0.9896±0.0009b
B	6878±961ab	3205±309ab	145.6±10.9b	9.25±0.13a	0.9937±0.0006a
BP	7694±840a	3507±240a	160.2±8.7a	9.39±0.16a	0.9940±0.0008a

Control: without biochar amendment; RC: with biochar amendment;

-P: without rape planting; +P: with rape planting.

Table S5 Correlations[a] of soil properties

		pH	moisture	TN	TC	C/N	NO_3^-	NH_4^+	CEC
pH	r	1	-	-	-	-	-	-	-
moisture	r	-0.188	1	-	-	-	-	-	-
TN	r	**0.909****	0.180	1	-	-	-	-	-
TC	r	**0.988****	-0.090	**0.956****	1	-	-	-	-
C/N	r	**0.998****	-0.214	**0.905****	**0.990****	1	-	-	-
NO_3^-	r	-0.148	**0.913****	0.193	-0.060	-0.173	1	-	-
NH_4^+	r	-0.513	-0.117	-0.533	-0.530	-0.496	-0.037	1	-
CEC	r	**0.891****	0.209	**0.938****	**0.910****	**0.876****	0.262	-0.538	1

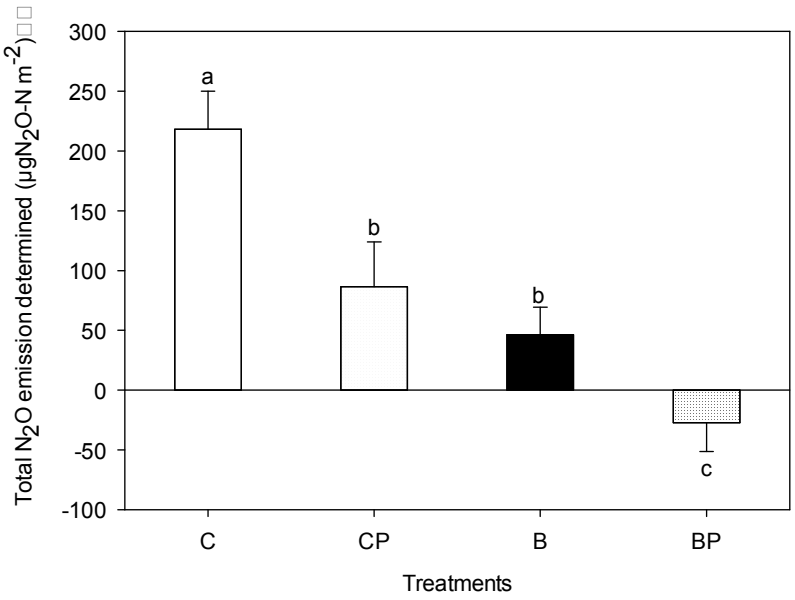
a: Pearson correlation coefficients were used to the correlation analysis. Difference were considered significant at a P value of $\leqslant$0.01(**).

Table S6 Correlations[a] of abundant phyla with soil properties

		Proteobacteria	Firmicutes	Acidobacteria	Chloroflexi	Actinobacteria	Gemmatimonadetes	Bacteroidetes	TM7	Cyanobacteria
pH	r	-0.073	0.480	**-0.947****	**-0.710****	0.293	**0.958****	**0.973****	**0.799****	-0.311
moisture	r	-0.526	0.331	0.226	0.027	-0.351	-0.279	-0.204	-0.264	0.360
TN	r	-0.329	**0.658****	**-0.840****	**-0.733****	0.104	**0.796****	0.906	**0.755****	-0.152
OC	r	-0.162	0.564	**-0.931****	**-0.738****	0.230	**0.912****	**0.973****	**0.825****	-0.271
C/N	r	-0.063	0.485	**-0.949****	**-0.718****	0.291	**0.946****	**0.974****	**0.826****	-0.308
NO_3^--N	r	**-0.638****	0.261	0.117	-0.021	-0.062	-0.204	-0.152	-0.263	0.503
NH_4^+-N	r	0.440	**-0.661****	0.426	0.145	0.105	-0.460	-0.503	-0.248	0.514
CEC	r	-0.235	0.495	**-0.817****	**-0.662****	0.204	**0.830****	**0.851****	0.601	-0.101

a: Pearson correlation coefficients were used to the correlation analysis. Difference were considered significant at a P value of $\leqslant$0.01(**).

147



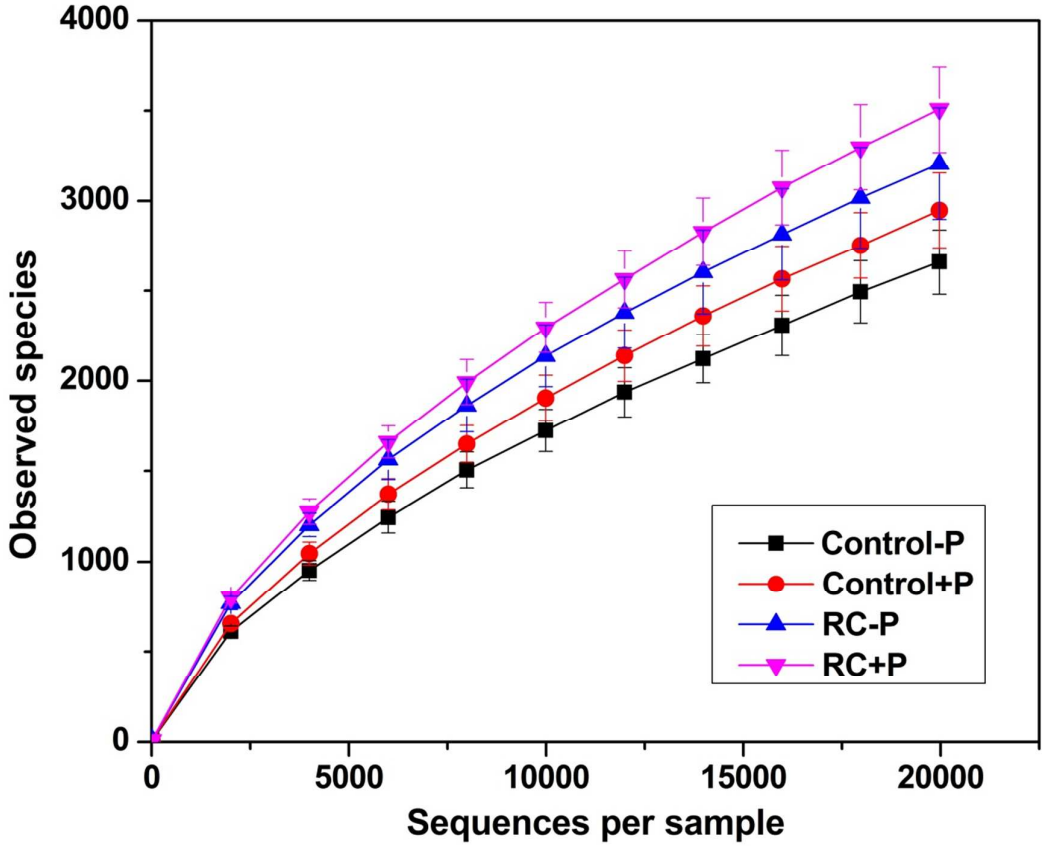
148

149 Figure S1 N_2O accumulation during determination period. The accumulation of N_2O

150 is calculated by adding N_2O emission in 2 hours determined each week. The letters

151 were used to show the differences among treatments.

152



153

154 Figure S2 Rarefraction of observed species at a sequencing depth of 19 772

155 sequences per sample

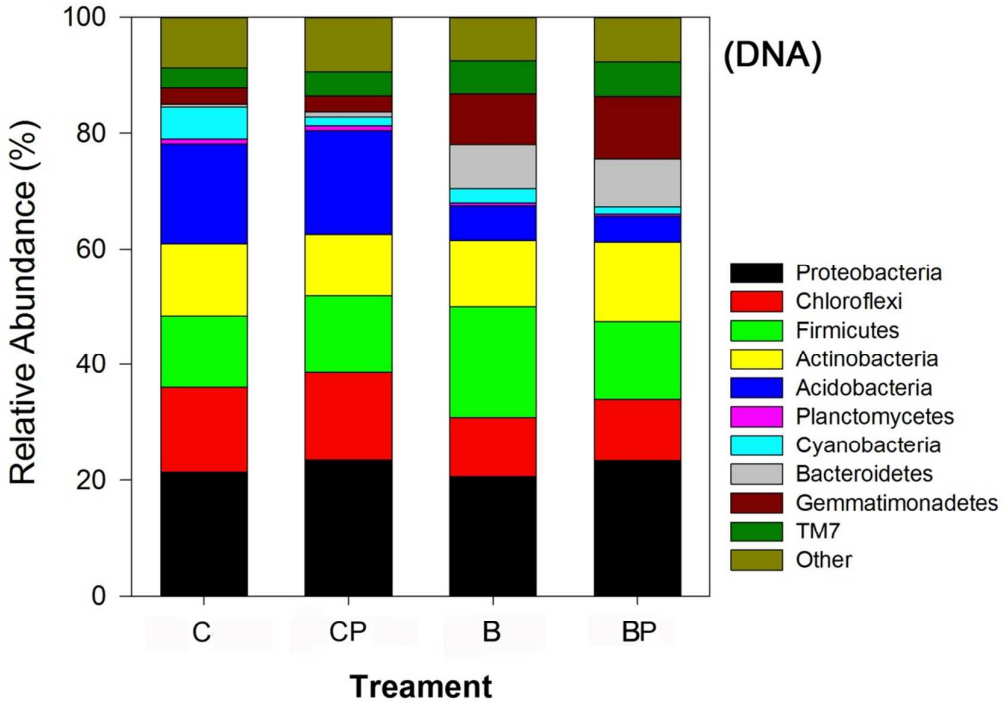
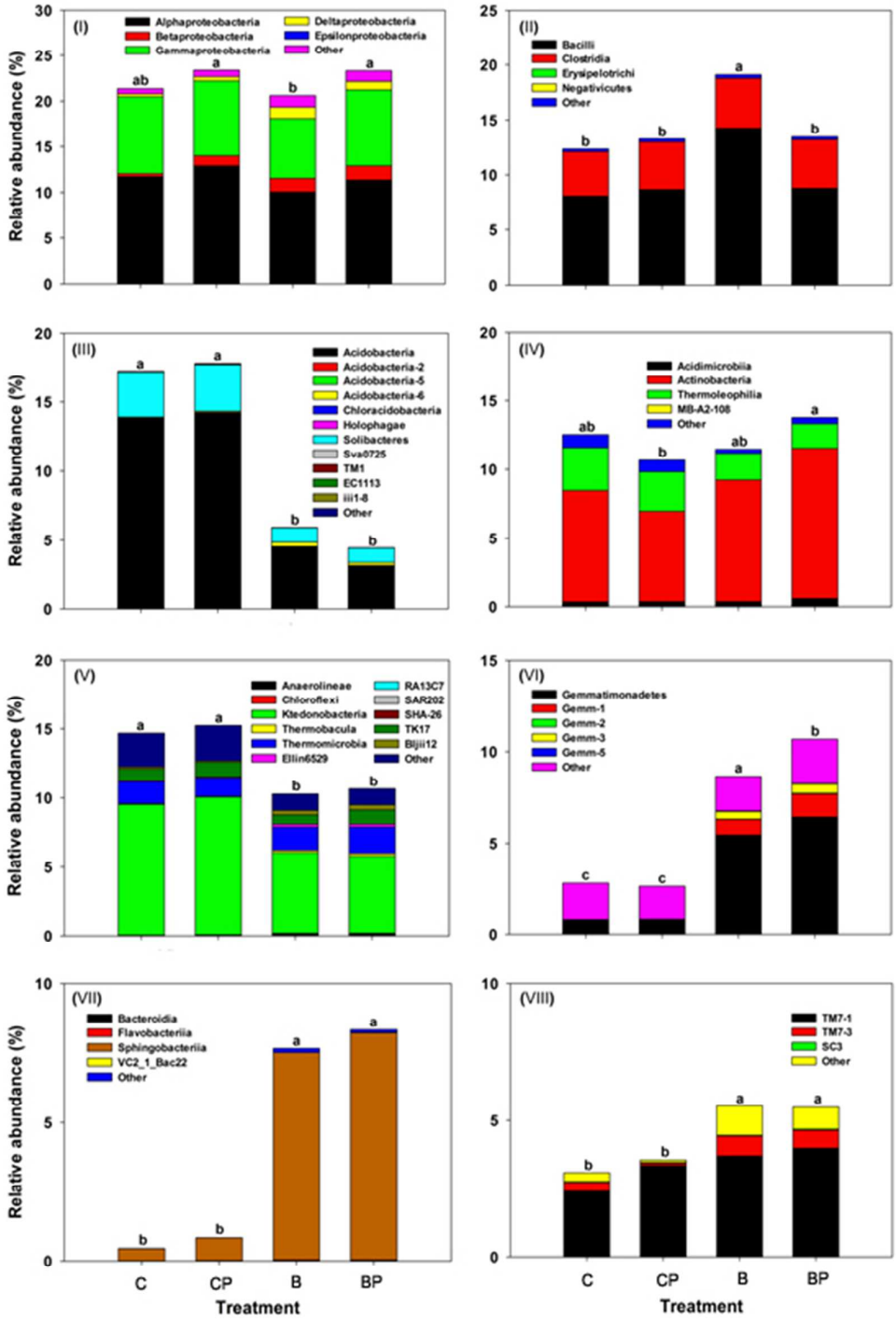


Figure S3 Relative abundances of bacterial community composition at phylum level detected in biochar treated and untreated soils. The abundance is presented in terms of an average percentage of four replicates, classified by RDP Classifier at a confidence threshold of 97%. “Other” refers to the sum of unclassified sequences and all other taxa with abundances lower than 0.9% in any sample. C：biochar untreated soil without rape, CP: biochar untreated soil with rape, B: biochar treated soil without rape, BP: biochar treated soil with rape.



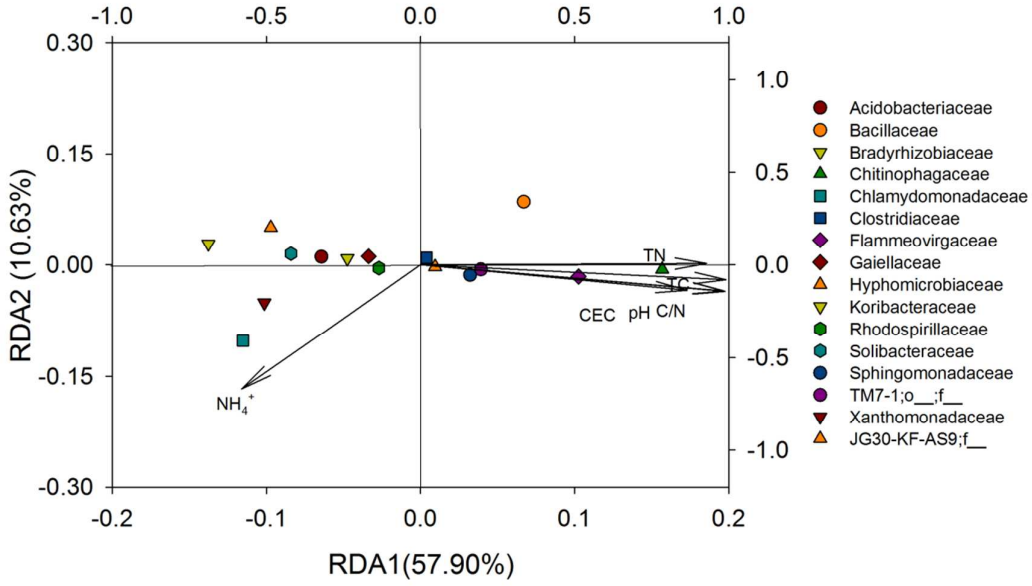
172

173 **Figure S4** Relative abundances of taxa at class level in the eight dominant phyla

174 detected in biochar treated and untreated soils (I: Proteobacteria, II: Firmicutes, III:

175 Acidobacteria, IV: Actinobacteria, V: Chloroflexi, VI: Gemmatimonadetes, VII:

176 Bacteroidetes, VIII: TM7). The abundance is presented in terms of an average
177 percentage of four replicates, classified by RDP Classifier at a confidence threshold
178 of 97%. “Other” refers to the sum of unclassified sequences and all other taxa
179 unnamed. C: biochar untreated soil without rape, CP: biochar untreated soil with
180 rape, B: biochar treated soil without rape, BP: biochar treated soil with rape. The
181 letters were used to show the differences among treatments at phylum level.



182

183 **Figure S5** Redundancy analysis (RDA) of bacterial community composition
184 obtained using the family abundances metrix in each sample. The symbols represent
185 different taxa in family level.

186 ## Supplementary references

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